Evolution of the capacity of bacteria-bacteria association to remove heavy metals from polluted soils

Submitted by

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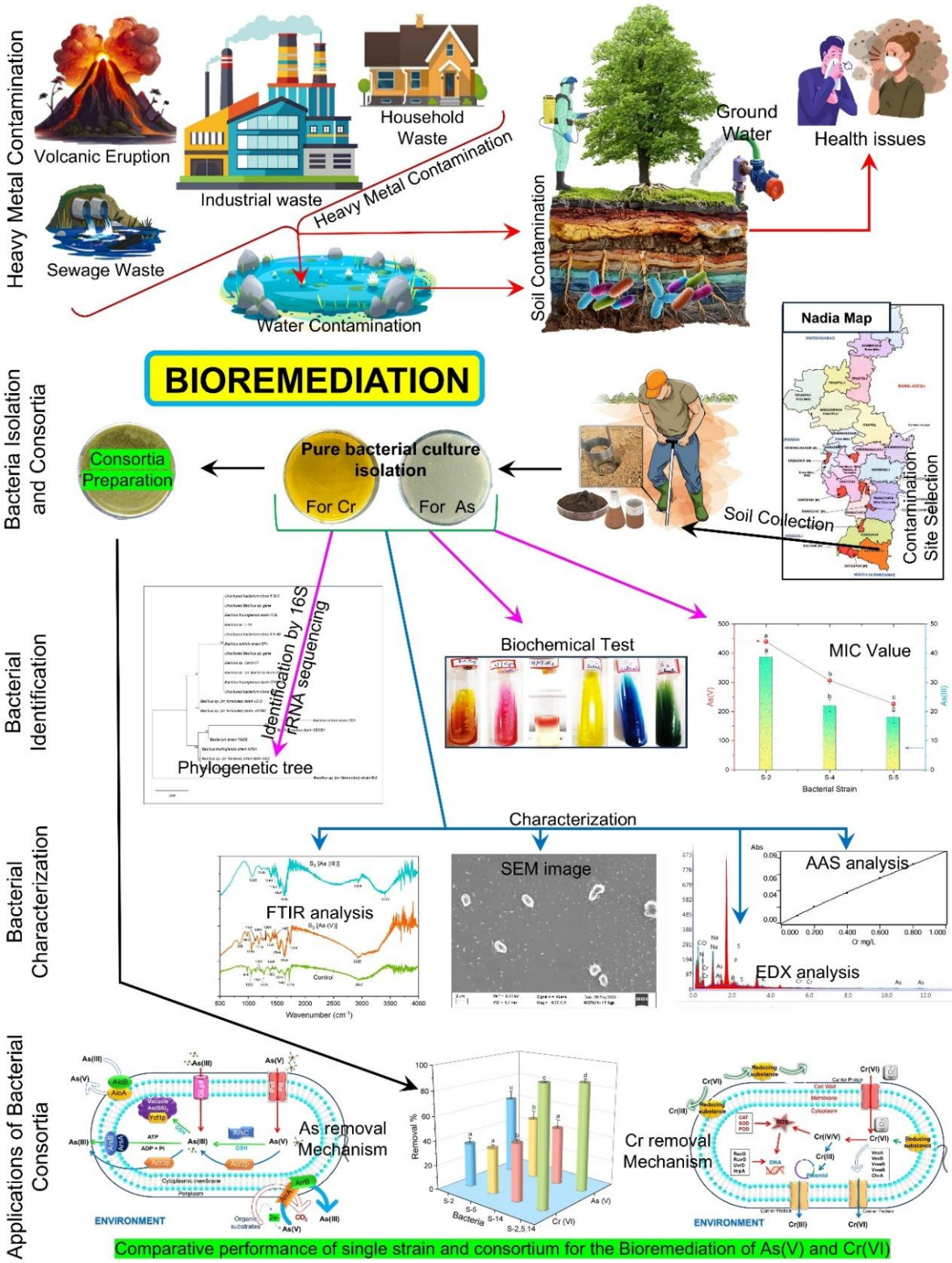
Graphical Abstract



Comparative performance of single strain and consortium for the Bioremediation of As(V) and Cr(VI)

Abstract

Heavy metal contamination poses significant environmental challenges, particularly in regions affected by industrial activities and agricultural runoff. This study reports the successful isolation, characterization, and identification of arsenic- and chromium-resistant bacteria from naturally contaminated soil in Mitrapur village, Nadia district, West Bengal, India, emphasizing their potential in bioremediation. The research highlights the potential of these indigenous bacteria for the eco-friendly bioremediation of arsenic As(V) and chromium Cr(VI) pollutants, along with their implications for environmental restoration.

The arsenic-resistant bacterial strain, identified as a rod-shaped, Gram-positive *Bacillus* sp. (*in: firmicutes*), has been deposited in the NCBI GenBank with accession number PQ061509. It demonstrated significant tolerance to arsenic, with minimum inhibitory concentrations (MIC) of 38 mM for arsenite As(III) and 440 mM for arsenate As(V). Biochemical assays, SEM image, and FTIR-based metabolic profiling revealed marked changes in bacterial metabolism under arsenic stress, offering valuable insight into arsenic resistance mechanisms. These findings highlight the strong adaptability of *Bacillus* sp. to arsenic pollution and its promise for arsenic bioremediation.

Similarly, the study also identified and evaluated chromium-tolerant bacterial strains from the same contaminated site. Strain S-5 exhibited flat to slightly convex colonies with white to light brown pigmentation, while S-14 formed round white colonies. Both strains were Gram-negative and exhibited high Cr(VI) resistance, with MIC values of 470 mM for S-5 and 453 mM for S-14. Based on 16S rRNA sequence analysis, S-5 was identified as *Achromobacter* sp. (NCBI Accession No PQ062229), and S-14 as *Enterobacter* sp. (Accession No PP993959). Morphological, biochemical, SEM image, and FTIR profiling confirmed these strains utilize multiple detoxification mechanisms, including enzymatic reduction of Cr(VI) to Cr(III), efflux transport systems, and mitigation of oxidative stress through antioxidant enzyme production. These native microbes show strong potential for affordable and sustainable chromium detoxification in polluted areas.

Significantly, the study explored the synergistic potential of a bacterial consortium comprising *Bacillus* sp. (S-2), *Achromobacter* sp. (S-5), and *Enterobacter* sp. (S-14) for the simultaneous remediation of arsenic and chromium. In aqueous systems, the consortium achieved 94% Cr(VI) and 87% As(V) removal within 48 hours, outperforming individual strains. In contaminated soil, bioaugmentation with the bacterial consortium resulted in 95% Cr(VI) and 82.9% As(V) removal after 100 days, whereas the non-bioaugmented control showed minimal removal. Kinetic modeling revealed that the degradation followed first-order reaction rates, with significantly shorter half-lives in the presence of the consortium, demonstrating enhanced detoxification efficiency due to microbial synergy.

Overall, this study emphasizes the effectiveness of well-characterized indigenous bacterial strains and consortia in bioremediation applications, offering a scalable, eco-friendly approach to mitigate heavy metal pollution, especially in regions impacted by industrial discharge and landfill leachate. The findings not only contribute to the growing body of knowledge on microbial resistance and detoxification strategies but also lay a strong foundation for future field-scale implementation. Further studies should focus on elucidating genetic resistance pathways, optimizing plant-microbe interactions, and enhancing environmental adaptability to fully harness these microbial resources for large-scale ecological restoration and sustainable agriculture.